HIV vaccine design and the neutralizing antibody problem

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Eliciting broadly neutralizing antibodies to human immunodeficiency virus could bring closer the goal of a successful AIDS vaccine. Here the International AIDS Vaccine Initiative Neutralizing Antibody Consortium discusses current approaches to overcome the problems faced.

Overcoming the problems of eliciting neutralizing antibodies (NAbs) to human immunodeficiency virus (HIV) will be no small task. Purely empirical approaches have thus far failed, as most recently demonstrated in two VaxGen efficacy trials of monomeric gp120. Years of research on the HIV-1 envelope (Env) glycoproteins have shown that the virus has many effective protective mechanisms with few vulnerabilities[1]. For fusion with its target cells, HIV-1 uses a trimeric Env complex containing gp120 and gp41 subunits (Fig. 1). The fusion potential of the Env complex is triggered by engagement of the CD4 receptor and a coreceptor, usually CCR5 or CXCR4. NAbs seem to work either by binding to the mature trimer on the virion surface and preventing initial receptor engagement events or by binding after virion attachment and inhibiting the fusion process[2]. In the latter case, NAbs may bind to epitopes whose exposure is enhanced or triggered by receptor binding.

Given the potential antiviral effects of NAbs, it is not unexpected that HIV-1 has evolved multiple mechanisms to protect it from antibody binding[3]. Env is heavily glycosylated, and the poorly or nonimmunogenic glycans prevents antibody access to the underlying peptide structure. Trimerization of the gp120-gp41 structure can shield vulnerable epitopes that are better exposed on the individual monomeric subunits, something still not fully understood at the molecular level but that seems to involve conformational rearrangements. Kinetic and spatial constraints impede antibodies from accessing potentially vulnerable sites during receptor binding and membrane fusion processes. Finally, the variable loops of gp120 are a prime target for NAbs, but such NAbs usually, although not always, have a very narrow breadth of reactivity and are not useful from a vaccine perspective.

Certain NAbs show Env has vulnerabilities

Despite all these defense mechanisms, primary isolates of HIV-1 from different genetic subtypes can be neutralized by some broadly reactive human monoclonal antibodies (mAbs) such as b12, 2G12, 2F5 and 4E10 (Table 1 and Fig. 1). In addition, a few rare sera from HIV-1-infected individuals have broad neutralizing activity. The very existence of these broadly neutralizing mAbs provides some hope that a vaccine inducing NAbs can indeed be created, by showing vulnerabilities in the viral defenses that could be exploited. An immunogen that could efficiently elicit NAbs with these same specificities would be a long way down the road to an effective vaccine. We believe, therefore, that it is important to understand how the mAbs described above interact with Env.

The first broadly neutralizing mAb identified was b12, an antibody that occludes the CD4 binding site on gp120 and prevents CD4 attachment . A key element of the CD4 binding site is a recess that forms a contact site for an amino acid, Phe-43, protruding from a loop on CD4 (ref. 1). Without this interaction, gp120 binds too weakly to CD4 for fusion to proceed efficiently. The b12 crystal structure shows that it has an unusually long protruding CDR3 loop that can plunge into the 'heart' of the CD4 binding site[4]. Many other mAbs with similar specificity have been identified over the years that block CD4 binding, although while they too bind to gp120 monomers in close proximity to the CD4 binding site, they lack the ability of b12 to neutralize primary HIV-1 isolates.

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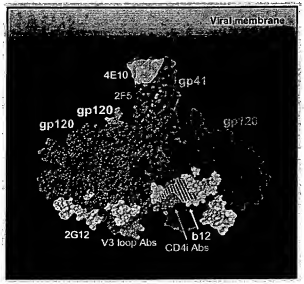

Figure 1 A model of the HIV-1 envelope spike showing the location of epitopes recognized by broadly neutralizing mAbs. The three gp120 monomers constituting the Env trimer are in shades of blue. The representation of gp41 in green is schematic. It is likely that the 4E10 and 2F5 epitopes are not available until the Env spike has engaged its cellular receptors. 'CD4i Abs' refers to the (approximate) epitope(s) for CD4-induced antibodies such as 17b and X5, and V3 loop antibodies to the (approximate) epitope(s) for antibodies to the tip of the V3 loop such as 447-52D. Diagram prepared by E.O. Saphire (Department of Immunology, The Scripps Research Institute, La Jolla, California) using published envelope spike models[20] (adapted from ref. 21 by permission of the publishers).

We still need to know more about why b12 is unique and what can be done to induce NAbs of the same specificity.

A second broadly neutralizing mAb, 2G12, recognizes a cluster of oligomannose residues on gp120 through a unique antibody structure[5]. In crude terms, 2G12 is shaped not like the traditional 'Y' but instead has its two arms pointing vertically and adjacent to one another. In molecular terms, a 'domain swap' of variable heavy chain domains has occurred to generate an antibody with three possible sets of antibody-combining sites in an array: two conventional heavy-light chain sites flank a heavy-heavy chain site. This array is well suited to recognize the cluster of oligomannose residues formed as part of the protective sugar coat of gp120. The antibody has, in effect, been raised against the very defenses that the virus uses to ward off antibodies in the first place. The relative conservation of a particular glycan arrangement allows 2G12 to recognize many HIV-1 strains. Once it binds, its bulk interferes nonspecifically with virus entry. Can sugar-based immunogens now be designed that allow the induction of 2G12-like NAbs?

The mAbs 2F5 and 4E10 recognize nearby but distinct epitopes on the membrane-proximal region of the gp41 ectodomain[6]. These NAbs do not interfere with receptor attachment but probably act by inhibiting the fusion process. The basic feature of the 2F5 epitope has long been known; it is superficially simple, frustratingly so, for it is apparently a simple linear peptide. Yet all attempts to induce 2F5-like NAbs by vaccination with peptides or simple linear epitopes have failed; the resulting antibodies recognize the immunogens but not the virus itself. Clearly, there is a critical, unusual aspect of the 2F5 epitope *in situ* on the Env complex that remains to be identified. Recent work indicates that 4E10 recognizes an ordered helical peptide structure. Additional studies are needed of both 2F5 and 4E10, preferably as complexes with more native Env structures, to show what immunization strategy might elicit NAbs with the same specificities. The mAb 4E10 in particular has a breadth of cross-reactivity that needs to be understood and exploited. The four mAbs discussed above are the most broadly neutralizing antibodies identified and validated in independent laboratories. More such antibodies may yet await discovery.

The V3 loop as a NAb target

Other antibodies have been described that have neutralizing activity in somewhat more circumscribed conditions. For example, the V3 loop of gp120 was once considered to be the 'principal neutralizing determinant', but this term applies only to viruses sensitized to NAbs by cell culture passage. The V3 loop is of more limited importance for primary virus neutralization, but some V3 mAbs are active against a range of primary viruses. One such mAb is 447-52D, which recognizes the GPGR motif at the tip of the V3 loop and main-chain atoms along one side of the loop[7] and neutralizes about 47% of clade B isolates. The antibody does not seem to recognize HIV-1 with a GPGQ motif at the tip of the V3 loop that is common to non-clade B isolates. Another antibody, 58.2, also recognizes an epitope overlapping the tip of the loop and neutralizes about 33% of clade B isolates. These V3 loop antibodies neutralize a similar set of viruses and are also reactive with monomeric gp120 from many viruses that they do not neutralize, indicating that the tip of the V3 loop may become inaccessible on Env from many primary isolates. An apparent homology has been reported between the V3 loop and a loop of similar length on CC chemokines, the 40s loop. Hence, it was suggested that the V3 loop structure evolved to mimic the 40s loop when interacting with the chemokine coreceptors CCR5 or CXCR4 (ref. 8). However, the involvement of the CC chemokine 40s loop in receptor binding is unclear because a large portion of the 40s loop is buried in the chemokine hydrophobic core and mutagenesis of the 40s loop region of the chemokines RANTES (CCL5) or MIP-1α (CCL3) does not affect chemokine receptor binding[9]. Future work will be required to determine whether HIV-1 gp120 mimics chemokines in binding the chemokine receptors and whether such structures can elicit NAbs efficiently.

The coreceptor site as a NAb target

The conserved coreceptor binding site is overlapped by the so-called CD4-induced epitopes that are typified by mAb 17b[1]. Immunoglobulin G (IgG) antibodies to the CD4-induced epitope cluster have very limited activity against primary isolates. Unexpectedly, however, Fab fragments and single-chain Fv fragments of mAbs to CD4-induced epitopes can neutralize primary isolates very well, in contrast to the normal pattern in which IgG molecules are more potent than Fabs[10]. In this case, the unexpected is also the unfortunate, because the immune system responds to antigens by raising whole antibodies, not fragments, and the whole antibodies lack neutralizing activity. Molecular modeling in association with crystallographic information shows the nature of the problem: kinetic and steric constraints. The CD4-induced epitope is formed only after gp120 has attached to CD4, and it points

Table 1 Cross-neutralizing properties of a panel of human mAbs to HIV-1

Virus	b12	b6	2G12	447-52D	58.2	X5	2F5	4E10
Clade B (total = 30)	22 (73%)	5 (17%)	22 (73%)	14 (47%)	10 (33%)	3 (10%)	24 (80%)	30 (100%)
All clades (total = 90)	45 (50%)	5 (6%)	37 (41%)	17 (19%)	10 (11%)	3 (3%)	60 (67%)	90 (100%)

Neutralization was considered positive if 50% neutralization was achieved at a mAb concentration of less than 50 μg/ml (half-maximal inhibitory dose of less than 50 μg/ml) in a relatively sensitive pseudovirus neutralization assay. b6 is a conventional mAb to the CD4 binding site; 58.2 is a mAb to the V3 loop, like 447-52D, directed to the tip of the V3 loop; X5 is a CD4-induced mAb. The other mAbs are described in the text. Data provided by J.M. Binley (Department of Immunology, The Scripps Research Institute, La Jolla, California).

down toward the cell membrane. Generally, it seems that either there is not enough room available for the bulk of an IgG molecule to fit into the gap between CD4, the Env complex and the coreceptor or there is not enough time for efficient binding to occur. In contrast, the smaller Fab fragments or a single-chain Fv molecule can bind to these sterically restricted and short-lived determinants[10].

Studies of mAbs to CD4-induced epitopes show another previously unknown aspect of the immune system's battle with HIV-1 (ref. 11). The conserved elements of the coreceptor binding site on gp120 interact with the N-terminal domain of CCR5 or CXCR4, on which two sulfated tyrosine residues are critically important. So how do some mAbs to CD4-induced epitopes see the same site on gp120? They do so by sulfating the tyrosine residues within their gp120 combining site, thereby mimicking the coreceptor N-terminal domain. This is an amazing solution to a tricky problem, but again, unfortunately, only a partial solution at best, as even the sulfated CD4-induced mAbs fall victim to the spatial and kinetic constraints imposed on antibodies by the geometry of the interactions between the virus and the cell surface[10,11].

Gp41: conserved but mostly inaccessible

Kinetic and steric constraints also probably protect potentially vulnerable regions of gp41 from NAb attack. The gp41 subunit is far more conserved than is gp120, and the fusion machinery is common to all strains. A peptide mimetic of a gp41 subdomain, enfuvirtide (formerly called T-20), is an effective, licensed antiviral drug. It works by intercalating within the fusion machinery and impeding necessary conformational changes. The regions of gp41 that correspond to enfuvirtide and the enfuvirtide binding site are highly immunogenic, yet antibodies to these sites do not neutralize primary viruses or even, for the most part, the sensitive, cell culture–adapted viruses. The reason for this is probably the limited space available for large antibody molecules to gain access to most gp41 epitopes when they transiently become exposed during the fusion process. The only gp41 regions shown so far to be accessible to NAbs at any stage are the 2F5 and 4E10 epitopes[6]. The frustration that this defense mechanism creates is exemplified by the realization that antibodies to relatively well-conserved gp41 epitopes can efficiently impede Env-mediated fusion when the fusion process is slowed by cooling of the reacting cells to 32 °C (ref. 12). But as we all know only too well, humans have a body temperature of 37 °C and at this temperature the same antibodies are relatively ineffective.

Strategies for immunogen design

How can we design immunogens that elicit broadly neutralizing antibodies? We see four principal strategies emerging that make use, to varying degrees, of what we have learned from the broadly neutralizing mAbs described above. The first strategy is to produce molecules that mimic the mature trimer on the virion surface. These molecules can be recombinant or expressed on the surface of particles such as pseudovirions or proteoliposomes[13–15]. The degree of mimicry can be estimated using the broadly neutralizing mAbs; good mimics will bind neutralizing mAbs well but non-neutralizing mAbs poorly. The second strategy is to produce Env molecules engineered to better present NAb epitopes than do 'wild-type' molecules. Deletion of variable loops[16] and elimination of carbohydrate attachment sites[3] are plausible approaches. An alternative approach is to try to reduce the immunogenicity of unwanted epitopes by, for example, hyperglycosylation or mutation, to attempt to focus responses to neutralizing epitopes[17]. The third strategy is to generate stable intermediates of the entry process with the goal of exposing conserved epitopes to which antibodies could gain access during entry. Immunization with CD4-ligated gp120, which has elicited broadly neutralizing NAbs in rabbits, is an example of this approach[18]. However, it is unclear what proportion of these NAbs are directed to CD4. The use of CD4 mimetic peptides in place of CD4 is a possible alternate method that is being studied. The fourth strategy is to produce epitope mimics of the broadly neutralizing mAbs determined from structural studies of the antibody-antigen complexes. The peptide epitopes recognized by 2F5 and 4E10 and the carbohydrate structure recognized by 2G12 are examples. All these approaches are being vigorously pursued, yet none has as yet efficiently elicited NAbs with broad specificity.

The Neutralizing Antibody Consortium

A common theme to the strategies described above is the interplay between structural biology, a better understanding of Env function and an appreciation of how antibodies engage native Env trimers. If we are to overcome the impressive defense mechanisms showed by Env, many of which have only been recognized in recent years, a highly collaborative, interactive and problem-solving approach to the neutralizing antibody issue will need to be taken. To address this, the International AIDS Vaccine Initiative has established the Neutralizing Antibody Consortium (NAC), comprising a flexible and expanding number of National Institutes of Health and extramural investigators with diverse backgrounds. This initiative, along with funding from the National Institute of Allergy and Infectious Diseases and the National Institute of General Medical Sciences, is predicated on the belief that by building on basic research advances through a coordinated and greatly expanded applied research agenda, progress toward solving the critical vaccine design problems will be accelerated.

The NAC is placing great emphasis on the determination of antibody and Env structures as a major starting point from which to apply rational vaccine design. In just the last 2 years, the structures of all the known broadly neutralizing antibodies, many with antigen (carbohydrate and peptides) bound, have been determined. However, we recognize that other important structural information remains to be obtained. In particular, we lack information on the structure of gp120 before CD4 binding: on gp120, including the variable loops, notably the V1, V2 and V3 loops; and on mature trimeric Env as it is found in functional spikes on the virus surface. We are attempting to accelerate structural studies by the use of advanced robotics developed for structural genomics for glycoprotein crystallization.

We also believe that candidate immunogens need to be rapidly and reliably tested in a comparative manner. Classically, academic laboratories tend to be good at the innovative phase of discovery, which in this context represents antigen design. However, such laboratories are less effective at the more repetitive but equally necessary tasks of immunogen evaluation. Furthermore, the end points here are the potency and breadth of neutralization by serum antibodies, which are very dependent on the assay used and the panel of isolates chosen. The NAC is establishing a generalized protocol to proceed from protein design to immunogen evaluation. Thus, Env proteins are prepared in one laboratory with standard protocols and quality control and are used to immunize rabbits at a contract laboratory. Serum neutralization assays are then done commercially with a panel of standard isolates chosen by the NAC. Rabbits are used for immunization because these animals can produce antibodies with long HCDR3 finger-like structures that have been linked to being important in the broadly neutralizing human mAbs b12, 2F5 and 4E10. Mice produce such antibodies very rarely. Finally, we are also seeking to accelerate progress by the sharing of results, 'know-how' and experience and by the generation of critical reagents in HIV vaccine research in bulk for communal use.

Concluding remarks

The neutralizing antibody problem is one of the most difficult of the many to be solved in the design of a successful HIV vaccine[19]. Its solution is likely to require considerable innovation in immunogen design, for which access to molecular information on broadly neutralizing mAbs and Env will be crucial. However, even with imaginative design, much effort will still be required in the evaluation of immunogens. We believe that the NAC approach represents a logical line of attack to the multiple challenges posed by immunogen design and evaluation at the heart of the neutralizing antibody problem. It offers, we suggest, a model for addressing other key scientific challenges that yet impede the development of an AIDS vaccine.

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